*A study of 461 children from December, 1957, to August, 1961, for antibody titers against enterobacterial pathogens revealed evidence of infection in about 25 per cent of the subjects. Differences in immunologic response to various pathogens were noted at different times. The data obtained indicate a high incidence of subclinical enterobacterial infections.*

# THE DETECTION OF ENTEROBACTERIAL INFECTION IN INSTITUTIONALIZED CHILDREN BY MEANS OF THE HEMAGGLUTINATION TEST

*Erwin Neter, M.D., F.A.P.H.A.; Albert H. Harris, M.D., F.A.P.H.A.; and Anne M. Drislane, M.D.*

ALTHOUGH modern public health measures have remarkably reduced the incidence of intestinal infections, diseases due to salmonellae, shigellae, and enteropathogenic Escherichia coli are still encountered, and even epidemics occur. It is also clear that mild and subclinical infections all too often escape detection, and that the available morbidity data do not give information on the total incidence of enteritis and colitis. The specific diagnosis of these enteric infections is made on a routine basis by the isolation of the etiologic agent from the feces of the patients or from other suitable specimens. The determination of the antibody response, such as the Widal test, has had only limited usefulness in the diagnosis of localized enterobacterial infections, in contrast to systemic typhoid fever. Extensive studies during the past decade have shown that the enterobacterial hemagglutination test is a sensitive procedure for the demonstration of enterobacterial antibodies.[1-15] The method is based on the fact that red blood cells readily take up the O antigens of enteric bacteria and thus become agglutinable in the presence of the corresponding bacterial antibodies. The method has proved to be considerably more sensitive than the conventional agglutination procedure. Furthermore, it is possible to modify erythrocytes with several antigens and thus have available a multivalent antigen for the demonstration of any one of the corresponding antibodies.[3] This procedure has proved useful for the demonstration of the specific antibody response in patients with bacteriologically proved enteric infection, and also for the diagnosis of subclinical infection in family members.[13] Another advantage of this serological procedure lies in the fact that enteric bacteria may not be recovered from a single stool specimen or may no longer be present several days after the onset of the infection at a time when a specific antibody response can be demonstrated. In view of these findings it was of interest

to determine whether subclinical or mild enterobacterial infection, not necessitating medical attention, can be diagnosed by this procedure in children housed in institutions. Such a field study was initiated in 1957. The results obtained indicate that subclinical enterobacterial infection occurred far more frequently than was suspected on the basis of clinical observation alone.

## Subjects, Materials and Methods

The subjects of the present study were children housed at three different institutions in New York State. Blood specimens were taken at four- (three to five) months intervals, from December, 1957, to August, 1961. Serum was kept frozen until used in the antibody titration studies. Titration of antibodies against salmonellae, shigellae, and enteropathogenic Escherichia coli was carried out by means of the multivalent and monovalent hemagglutination tests as follows.

Smooth strains of the enteric bacteria were grown on brain veal agar in Kolle flasks at 37° C for 18 hours. The resulting growth was suspended in 25 ml of phosphate hemaggultination buffer (Difco; pH 7.3). The suspension was heated in boiling water for one hour. The supernate obtained after centrifugation at 23,500 G was kept frozen until used.

Multivalent salmonella antigen was prepared by mixing supernates from representative strains of group B, C1, C2. D, and E, each in a final dilution of 1:10. Similarly, multivalent antigens were prepared from S. flexneri, S. boydii and S. sonnei, representing five different serotypes and from enteropathogenic Escherichia coli, including serogroups O26, O55, O111, O127, and O128. Thus, three multivalent antigens were employed. Because of the prevalence of S. sonnei, monospecific hemaggultination tests were carried out on all specimens. In addition, whenever, indicated, all five antigens present in a multivalent antigen were used singly, also in a final dilution of 1:10.

Human erythrocytes obtained from healthy subjects of blood group O were modified as follows. Red blood cells in a concentration of 2.5 per cent were washed three times. To the sediment the respective antigen was added to a final erythrocyte concentration of 2.5 per cent. The mixture was then incubated in a waterbath at 37° C for 30 minutes. The red cells were again washed three times with phosphate buffer, in order to remove excess antigen.

The hemagglutination test was carried out as follows. Serum in a volume of 0.2 ml, in serial twofold dilutions, was mixed with equal amounts of antigenically modified erythrocytes. The mixtures were incubated in a waterbath at 37° C for 30 minutes. Hemagglutination was read after centrifugation at 1,300 G for two minutes. All serum specimens from a single individual were titrated simultaneously. In addition to the multivalent hemagglutination test serum specimens with significant titers were tested for antibodies against each of the serogroup of enteric bacteria included in the multivalent antigen. Thus, it was possible to determine whether a given high titer of E. coli antibodies in the multivalent test was due to antibodies against groups O26, O55, O111, O127, or O128.

An antibody response was considered to be present if at least a fourfold rise in antibody titer occurred against a single serogroup of the enteric pathogens, in the absence of a similar change in titers against all others.

## Results

Between December, 1957, and August, 1961, an immunologic study on the presence of antibodies against enterobacterial pathogens was carried out

**Table 1—Ages of Subjects at Initiation of Study**

| Institutions | Ages of Subjects | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0–1 | 1–2 | 2–3 | 3–4 | 4–5 | 5–6 | 6–7 | 7–8 | 8–9 | 9–10 | 10–11 |
| | Number of Subjects | | | | | | | | | | |
| W | 3 | 16 | 45 | 96 | 98 | 10 | 0 | 1 | 1 | 1 | |
| J | 6 | 5 | 13 | 9 | 6 | 17 | 11 | 16 | 10 | 10 | 4 |
| R | 7 | 7 | 21 | 18 | 26 | 3 | 0 | 1 | | | |

on 461 children housed in three different institutions. Table 1 gives information on the ages of the children upon entry into this study. A total of 3,140 blood specimens, taken at approximately four-month intervals, were available for this investigation. Information on the average number of blood specimens per subject is presented in Table 2.

Illustrative of the evidence obtained of enterobacterial infection by the study of the immunologic response of these subjects are the data obtained on a child in home W, as summarized in Table 3. It can be seen that between August and December, 1960, the antibody titer against Shigella sonnei showed a striking increase, from 1:20 to 1:320, whereas the titers of the antibodies against five serogroups of salmonellae and five serogroups of enteropathogenic Escherichia coli remained constant. Further, the titers of antibodies against S. sonnei had remained at the same level from April. 1958, to August, 1960. It is this monospecific antibody rise that has been utilized in the entire series as evidence of an immunologic response for the presumptive diagnosis of infection with the respective pathogen.

The immunologic data are summarized in Table 4 and are based on a conservative interpretation of "significant" antibody responses to various enteric pathogens, as mentioned above.

It can be seen that during the entire period of observation approximately 15 per cent of the subjects had a significant antibody response to Shigella sonnei, 2 per cent to other shigellae, 4 per cent to salmonellae, and 4 per cent to enteropathogenic Escherichia coli, amounting to 25 per cent for all pathogens. It must be realized that all subjects were not studied for the same length of time; approximately eight serum specimens per subject (over a pe-

**Table 2—Number of Serum Specimens per Subject Tested for Enterobacterial Antibodies**

| Institutions | Number of Serum Specimens | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| | Number of Subjects | | | | | | | | | | | |
| W | 8 | 6 | 15 | 9 | 16 | 26 | 16 | 28 | 64 | 83 | | |
| J | 0 | 28 | 10 | 19 | 14 | 36 | | | | | | |
| R | 0 | 5 | 6 | 12 | 4 | 8 | 5 | 7 | 8 | 10 | 14 | 4 |

**Table 3—Titers of Antibodies of Subject W153 Against Various Enteric Pathogens**

| Dates of Serum Specimens | Pathogens | | |
|---|---|---|---|
| | Shigella sonnei | Salmonella Groups B to E | Entero-pathogenic Escherichia coli |
| | Antibody Titers (Reciprocal) | | |
| April 12, 1958 | 20 | 40 | 40 |
| August 18, 1958 | 40 | 40 | 40 |
| December 13, 1958 | 40 | 40 | 40 |
| April 27, 1959 | 20 | 40 | 40 |
| August 7, 1959 | 20 | 40 | 40 |
| February 19, 1960 | 20 | 40 | 40 |
| August 12, 1960 | 20 | 40 | 40 |
| December 12, 1960 | 320 | 40 | 40 |
| April 13, 1961 | 320 | 40 | 40 |
| July 27, 1961 | 80 | 20 | 20 |

riod of two and a half years) were available from series W and only 4 per subject (one and a quarter years) for series J. It is of more than passing interest that the immunologic evidence of enterobacterial infection shows rather significant differences between the three series. For example, evidence of Shigella sonnei infection was found more frequently in series R than in series W and J. Conversely, evidence for infection due to other shigellae and enteropathogenic Escherichia coli was encountered more frequently in series W than in series R and J. It is of interest to point out, too, that Shigella sonnei infection in series R was not equally distributed throughout the entire period of observation. Only two subjects with this infection were encountered in the nine-months period from October, 1959, through June, 1960, whereas 20 cases were documented between July, 1960, and March, 1961. Although in 1958 clinical illness occurred due to Shigella flexneri, diarrhea, necessitating bacteriologic studies, was not encountered in the subjects with immunologic response to enterobacterial pathogens later in the study. It is evident, therefore, that based

**Table 4—Subjects with Significant Enterobacterial Antibody Response**

| Institutions | Total Number of Subjects | Total Number of Serum Specimens | Average Number of Serum Specimens per Subject | Pathogens | | | |
|---|---|---|---|---|---|---|---|
| | | | | Shigella sonnei | Other shigellae | Salmonellae | Enteropathogenic Escherichia coli |
| | | | | Number of Subjects and Approximate Percentages | | | |
| W | 271 | 2,082 | 8 | 47 (17%) | 10 (4%) | 17 (6%) | 18 (7%) |
| J | 107 | 448 | 4 | 1 ( 1%) | 0 (0%) | 0 (0%) | 0 (0%) |
| R | 83 | 610 | 7 | 22 (27%) | 0 (0%) | 2 (2%) | 0 (0%) |
| Totals | 461 | 3,140 | 7 | 70 (15%) | 10 (2%) | 19 (4%) | 18 (4%) |

on this immunologic study, probable infection with various enteric pathogens in these institutionalized children occurred more frequently than was apparent from clinical observation alone.

## Discussion

Intensive studies of the basic aspects of the enterobacterial hemagglutination test have been carried out in our laboratories and in other institutions since 1952.[1-15] As a result, it has been established that this serologic technic, when combined with proper interpretation and consideration of the common antigen of Kunin discussed below, provides a sensitive and specific tool for measuring the antibody response of human subjects and of animals to various enteric pathogens. It was only after these basic studies had been completed that the field study, reported here, was undertaken.

The present investigation has revealed that in institutionalized children immunologic evidence of enterobacterial infection was obtained more frequently than was anticipated from clinical observation alone. It is well known that Shigella, Salmonella, and enteropathogenic Escherichia coli infection may be either subclinical, mild, or severe. It is particularly with regard to these mild or subclinical infections that immunologic studies can provide appropriate information for etiologic diagnosis. Further, information thus gained contributes to a more complete knowledge of the incidence of these infections (serologic or immunologic epidemiology).

Immunologic studies, such as the one reported here, have the advantage of documenting a specific antibody response at a time when the pathogen may no longer be present or may not be isolated for other reasons. On the other hand, it is important to emphasize that the antibody titer decreases from its peak within 3 to 12 months and that immunologic studies yield information only on present or relatively recent infection.[6]

Finally, it must be emphasized that the hemagglutination test, as employed here, measures not only the group specific O antibodies, but antibodies against an antigen common to many enteric bacteria as well. This antigen was discovered by Kunin, et al., and has been studied extensively in our laboratories.[16-18] Antibodies against the common antigen can be eliminated by appropriate inhibition tests. The presence of this antibody usually does not interfere with appropriate interpretation of the results, unless it is present in high titer and then responsible for agglutination of red cells modified by all or most enterobacterial antigens. A few such instances have been observed in the present study and are excluded from the group of probable infection.

In this field study, immunologic evidence was obtained for subclinical enterobacterial infection in approximately 25 per cent of institutionalized children studied over a period of three and a half years.

## Summary

A study was made from December, 1957, to August, 1961, on 461 children in three institutions (3,140 blood specimens) with regard to antibody titers against enterobacterial pathogens. Based on conservative interpretation of the multivalent and monovalent enterobacterial hemagglutination tests, evidence of infection was obtained in approximately 25 per cent of the subjects. Striking differences were noted in the immunologic response against different pathogens in the three series and also at different times during the investigation. In view of the absence of significant clinical illness, the data obtained provide information on the un-

expectedly high incidence of subclinical enterobacterial infections.

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Dr. Neter is professor of clinical microbiology, State University of New York at Buffalo, and director of bacteriology at Children's Hospital, Buffalo, N. Y. Dr. Harris is assistant director, Division of Laboratories and Research, New York State Department of Health, Albany, N. Y. At the time of the study Dr. Drislane was assistant director, Bureau of Maternal and Child Health, New York State Department of Health, Albany, N. Y.

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